Claims

We claim:

1. A method of producing human coagulation factor VIII, comprising:

(a) subcloning an encoding sequence into a plant expression vector and obtaining a subcloned plant expression vector;

b) transferring the subcloned plant expression vector into a plurality of plant cells;

(c) selecting a plurality of positive transformants from the plurality of plant cells on an antibiotic selective media;

(d) growing the plurality of plant cells in whole plants or suspensions; and

(e) extracting and purifying the human coagulation factor VIII from the plurality of transgenic plant cells.

2. The method as recited in claim 1, wherein transferring is by direct particle bombardment.

3. The method as recited in claim 1, wherein transferring is by agrobacterium mediated transformation.

4. The method as recited in claim 1, wherein transferring is by pollen transformation.

5. The method as recited in claim 3, wherein agrobacterium mediated transformation comprises the steps of:

(a) placing the subcloned plant expression vector to an agrobacterium;

(b) co-cultivating the agrobacterium containing the subcloned plant expression vector with the plurality of plant cells.

6. A method of producing a human coagulation factor VIII from plant cells, comprising the steps of:

(a) introducing an encoding sequence for production of human coagulation factor VIII into a plant expression vector in the plant cells;

(b) obtaining a positive transformant of the plant cells, the positive transformant carrying genetic material encoding the production of the human coagulation factor VIII;

(c) cultivating the positive transformant; and

(d) obtaining the human coagulation factor VIII.

7. The method as recited in claim 6, wherein said encoding sequence is selected from the group consisting of copy DNA, genomic DNA and combinations thereof.

8. The method as recited in claim 6, wherein factor VIII is cultivated in the whole plant.

9. The method as recited in claim 6, wherein factor VIII is cultivated in a plant tissue culture.

10. The method as recited in claim 6, wherein factor VIII is extracted and purified by a process selected from the group consisting of protein precipitation, ultrafiltration, affinity chromatography, and electrophoresis.

11. The method as recited in claim 6, wherein said human coagulation factor VIII is an amino acid sequence substantially that of human coagulation factor VIII.

12. The method as recited in claim 6, wherein said encoding sequence encodes a full length of said human coagulation factor VIII .

13. The method as recited in claim 6, wherein said encoding sequence encodes a full length of said human coagulation factor VIII deleting the B-domain.

14. The method as recited in claim 6, wherein said encoding sequence encodes a full length of human coagulation factor VIIIa .

15. The method as recited in claim 6, wherein said encoding sequence encodes human/porcine factor VIII wherein the A2 epitope of human factor VIII is replaced with the analogous porcine sequence.

16. The method as recited in claim 6, wherein said encoding sequence encodes factor VIII heavy chain protein.

17. The method as recited in claim 6, wherein said encoding sequence encodes factor VIII light chain protein.

18. The method as recited in claim 6, further comprising modifying the encoding sequence by adding a regulatory element selected from the group consisting of leader sequences, signal peptides, transcription promoters or enhancers, and transcription terminators.

19. The method as recited in claim 6, wherein said encoding sequence is provided by adding said transcription promoter to the upstream or 5' end of the encoding sequence; and adding said transcription terminator to the downstream or 3' end of the encoding sequence.

SUB B5

20. The method as recited in claim 19, further comprising adding an additional regulatory element encoding a signal peptide, said additional regulatory element added between the transcription promoter and the upstream 5' end of the encoding sequence.

21. The method as recited in claim 20, further comprising adding a regulatory element encoding an untranslated leader sequence between the transcription promoter and the additional regulatory element encoding the signal peptide to enhance mRNA stability.

22. The method as recited in claim 20, further comprising adding a regulatory element encoding an untranslated leader sequence at the downstream or 3' end of the encoding sequence to enhance mRNA stability.

SUB B6

23. A method of producing a human coagulation factor VIII using an agrobacterium mediated transformation, comprising:

(a) modifying a coagulation factor VIII encoding sequence for subcloning into a plant expression vector;

(b) subcloning the encoding sequence into the plant expression vector;

(c) transferring the plant expression vector to agrobacterium;

(d) co-cultivating a portion of the transgenic plant cells with the agrobacterium;

(e) selecting positive transformants from the co-cultivated culture on an antibiotic selective media;

(f) permitting growth of the transgenic plant cells in whole plants or suspensions; and

(g) extracting a quantity of human coagulation factor VIII from the plant cells.